Donor tissue for corneal transplantation

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Chapter 5

Quality aspects in eye banking

Chapter 5.1

Different eye banks for tissue processing and graft outcome: trust and control

submitted

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Abstract

Purpose

To compare graft outcome when different eye banks are involved in the processing of organ cultured donor tissue.

Methods

Donor tissue was collected in the Netherlands by one organisation. It was preserved and processed by organ culture technique in 5 different eye banks. Grafting was performed by 5 experienced surgeons. The National Cornea Follow-up Registry was the source for the prospectively collected follow-up data.The study group consisted of 173 penetrating grafts performed in the period 2002-2003.The results were compared with a historical group of grafts performed by the same surgeons in the period 1995-2001 and matched for diagnosis, recipient age and gender. Outcome results were: primary failure, delayed epithelialisation of the graft (> 9 days), graft clearness, graft survival and donor related infections. Variables analysed for effect on clinical outcome were: year of surgery, surgeon, eye bank, recipient age and gender, previous grafts in the same and fellow eye, pre-operative diagnosis and degree of vascularization of the recipient cornea.The composition of the 2 groups was compared using the chi-square and ANOVA test. Odds ratios were assessed for the risk of primary graft failure, delayed epithelialisation and graft clearness. Multivariate logistic regression analysis was applied to detect variables affecting graft outcome. For graft survival the Kaplan Meier and log rank tests were used.

Results

For the study group and the historical group the odds ratio estimate for delayed epithelialisation of the graft was 3.4 (p=0.019)and for graft clearness 6.7 (p <0.0001) respectively. Five cases of PF were observed in the study group compared to 0 in the historical group. The eye bank turned out to be a risk factor for graft clearness and delayed epithelialisation of the graft when study and historical group were combined. Graft survival for a 2 year period was different for the eye banks (p=0.005).There were no cases of donor related infections.

Conclusion

Graft outcome may be affected by the eye bank processing the donor tissue. Trust in eye banks can be enhanced by control based on the registration of follow-up data and analysis of trends.

Key words

Eye banks- corneal graft outcome- organ culture- penetrating keratoplasty.

Introduction

In Europe, organ culture is the commonly used preservation method.[1] This method facilitates exchange because of the long term storage and mandatory evaluation of the endothelium at the end of the storage period. Nevertheless experience with exchange is very limited (7%).[2] Common practice for corneal surgeons in Europe has been to trust the local eye bank with the exception of HLA typed corneas.[3] The effect of HLA matching on organ cultured graft survival has been studie.[4,5,6,7] The tissue was derived from different banks but this was not an issue.

There are few publications about international donor sharing.[8,9] Some authors [8,10] concluded that foreign donor corneas were as effective and safe as domestic donor corneas while others[9] reported high endothelial cell loss after transport and relatively low cell counts 15 months after surgery. These studies are about hypothermically preserved (Optisol) corneas.

The corneal surgeon cannot rely on experimentally supported arguments to judge the suitability for grafting of a stored corneoscleral disc; studies about the effect of different technical aspects of eye banking on graft outcome are still lacking.

In 2002-2003 a unique situation existed. The activities of the local eye bank were seriously reduced due to organisational reasons. Donor tissue retrieved in the Netherlands was processed in different eye banks, claiming the same storage technique (organ culture) and selection criteria.[2] In this way the donor tissue chain was saved and grafting could be continued. In the Netherlands the allocation of tissue is legally performed by one organisation, and the corneal surgeons were not aware that other banks were involved in processing. Clinical outcomes of grafting in the Netherlands were prospectively collected since 1995 by all Dutch surgeons in the National Cornea Follow-up Registry.These circumstances provided the possibility to study the effect of eye banks on the outcome of grafting.

In addition, during 2002-2003, rumours raised amongst the surgeons about poorer graft outcome ascribed to donor tissue quality. It became generally known that other eye banks than their well known local eye bank had been processing the tissue. This resulted in refusals to accept tissue from certain eye banks. These refusals triggered this study.

To find scientific support for the rumours, at first the outcome of grafts in the period 2002-2003 has been evaluated and compared to a historical group of grafts performed in the period 1995-2001. Secondly, risk factors for poor graft outcome were analysed.It will be discussed whether the presence of a reporting mechanism for serious adverse reactions, as required in the EU directives,[11,12,13] would have facilitated the detection of differences in graft outcome.

Material and Methods

Between 1-1-2002 and 31-12-2003 619 elective penetrating grafts were performed in the Netherlands. In order to reduce the number of involved confounding variables the following selection criteria were used to form the study group:

Patients with mental disability were excluded because of difficulties with physical examinations.

Selection of surgeon was based on the following items: The surgeons were trained in the same centre. They had a minimum of 5 years experience and performed at least 25 keratoplasties each year. Result: totally 5 surgeons, 4 centres, 291 graft procedures.

Selection of banks: Eight banks processed the donor tissue. Banks providing more than 20 corneas were included, resulting in 4 banks.

As a result of these criteria the study group consisted of 173 grafts. To get a historical group for comparison, a group of 173 grafts performed by the same 5 surgeons in the preceding period (1995-2001) were selected. The selection was based on the surgeon and diagnosis, gender, age of the recipient (see table 1).

As far as prospective data were not yet complete, they were collected retrospectively by examining the medical records. In those cases the origin of the cornea was masked for the investigator.

Donor tissue

Human eyes were donated to the Netherlands Transplantation Society (NTS) for transplantation purposes. According to the national law NTS was responsible for the screening of the donors. This included screening of the medical history and serology testing. NTS delegated its responsibilities for donor tissues to Bio Implant Services Foundation (BIS). Eye retrieval by specifically trained staff was organized by BIS. The globes were stored in a moist chamber. Transport of the tissue to the eye banks and from the eye banks to the clinic was also organised by BIS. Selection, processing and storage in OC in the different eye banks were performed according to the general technical standards of the European Eye Bank Association (EEBA).[2] Variations in technical details however existed.[14]

In the study period the activities of the national eye bank were seriously reduced for organisational reasons and the donor tissue was distributed to different eye banks for processing.

Surgical methods

The different corneal surgeons performed standard surgical techniques to perform the penetrating keratoplasties.[15] The grafts survival of the 5 surgeons did not significantly differ in the study period, the historical period and in the period after the study (The National Cornea Follow-up Registry, non published results).

Table 1 - Demography and matching characteristics of the study group and the historical control group

Covariates		*OK-period*	*test*	*P-value*	
		number			
		2002-2003	*1995-2001*		
Recipient	Mean age	63	59	x^2	0.031
	Median age	69	64		
	< 30 years	11	14	Anova	.223
	30-49 yrs	29	37		
	50-69 yrs	50	58		
	> 70 yrs	83	64		
	Gender male	91	87	x^2	.373
	Previous graft this eye	29	28	x^2	1.000
	Previous graft other eye	36	29	x^2	.409
	Vascularization	9	12	x^2	.653
Diagnosis	Keratoconus	25	22	x^2	
	Herpes	13	12		
	Fuchs'	47	48		.302
	BKP	32	35		
	Regraft	22	17		
	Miscellaneous	34	39		
Surgeon	1	28	30	x^2	
	2	11	11		
	3	66	60		.976
	4	40	42		
	5	28	30		
Bank	1	29			
	2	25			
	3	87			
	4	32			
	5		173		
Totals		173	173		

National Cornea Follow-up Registry

In 1995 a National Cornea Follow-up Registry was started. Data were prospectively collected in a standardised way with forms, filled in by the surgeons. Pre- and peroperative information was complete in 98% of the cases, follow-up data (at least one follow-up visit after transplantation) were received for 78% of the grafts.

Outcome results

As outcome results were considered: primary failure,[16,17,18] delayed epithelialisation of the graft, graft clearness, graft survival and donor related infections.
Primary graft failure was defined as a graft which never cleared up after transplantation.[18] The moment of this measurement was ultimately 6 months postoperatively.
A delayed epithelialisation of the graft was defined in the study as an epithelial defect that still existed 9 days postoperatively.
Graft clearness was graded by the surgeon as crystal clear (grade I), clear (grade II), partially hazy (grade III) and cloudy (grade IV). Grade III and grade IV were considered as an event for the graft survival.The date of failure was defined as the first postoperative examination date the patient was seen with a graft, graded grade III or grade IV.
Survival curves were calculated using the actuarial life table method by Kaplan-Meier. Infections after transplantation, which could be attributed to the donor, were described as donor related infections.

Statistics

Frequencies of the covariates in the study and historical and control group were compared with Chi-square test. Differences in means were calculated with ANOVA test. Risks in outcome in the study group and the historical group were compared with the odds ratio estimate (95% confidence interval). Multivariate logistic regression analysis was performed to identify risk factors for outcome. Differences in graft survival between classes were assessed with a log rank test. The SPSS software (version 16.0) was used. P values less than 0.05 were considered statistically significant.

Results

The results of 346 keratoplasties with cornea tissue processed in 5 different eye banks were evaluated. In the period 2002-2003, 173 corneas were transplanted with tissue from 4 different banks and in the period before (1995-2001) 173 with tissue from 1 eye bank.
The 2 groups were comparable for the covariates gender, previous graft in the operated eye, previous graft in the fellow eye, vascularisation, diagnosis and surgeon. The age of the patients operated in the period 2002-2003 was slightly more senior than in the period 1995-2001 (Table 1).

Clinical outcomes in the 2 different periods

Primary graft failure, Delayed epithelialisation of the graft, Graft failure, Donor related infections

Five cases of primary failure were reported in the study period, 2 x after 1 month, 2 x after 2 months and 1 x after 5 months. In the historical period no primary graft failures were reported. For primary graft failure the odds ratio could not be calculated (Table 2). Primary failure was observed in 4 cases with corneal tissue from bank 3 and in 1 case with tissue from bank 4 (Table 3).

Table 2 - Clinical outcome results, numbers, odds ratio estimate and 95% confidence ratio for the study period (2002-2003) and the historical period (1995-2001)

	Numbers		*Odds ratio*		
Outcome results	*2002-2003*	*1995-2001*	*estimate*	*95% confidence limits*	*p-value*
Primary graft failure	5	0	*		0.072
Delayed epithelialisation of the graft	16	5	3.424	1.226 – 9.567	0.019
Graft failure	29	5	6.767	2.553 – 17.937	< 0.0001

Test common odds ratio equals 1
* cannot be calculated, one field value zero.

Table 3 - Primary corneal graft failure, delayed epithelialisation of the graft for the 5 different eye banks

		Primary failure	*%*	*Delayed epithe-lialisation*	*%*	*Graft clearness not clear*	*%*
Bank 1	29	0	0	2	6.9	2	6.9
Bank 2	25	0	0	6	24.0	4	16.0
Bank 3	87	4	4.6	6	6.9	15	17.2
Bank 4	32	1	3.2	2	6.3	8	25.0
Bank 5	173	0	0	5	2.9	6	3.5

A delayed epithelialisation of the graft was reported in 16 cases in the study period and in 5 cases in the period 1995-2001, an increased risk of 3.4 x (table 2). A delayed epithelialisation of the graft was observed in 24% of cases with tissue derived from bank 2 (table 3).Graft failure was reported in 29 cases in the study period and in 5 cases in the historical period, an increased risk of 6.7 x (table 2).

Clinical outcomes were different for both delayed epithelialisation of the graft (p=0.019) and graft failure (p < 0.0001) as outcome result. Donor related microbial infections were not reported in any of the grafts, in the study as well as in the control group.

Graft Survival
Kaplan Meier survival curve showed a difference for the study period and the historical period (p=0.040).

Variables affecting clinical outcomes

A multi variate stepwise logistic regression analysis was performed to detect the variables affecting the risk of primary graft failure, delayed epithelialisation of the graft and graft clearness in the study group, the historical group and when study and historical group were combined. The Chi-square of the omnibus test was low for both the study and historical group. Statistically reliable risk factors could not be determined. For the overall group the Chi-square of the omnibus test was reliable(p= 0.046). For the outcome delayed epithelialisation of the graft en graft clearness the eye bank was a significantly risk factor (p= 0.007 and p= 0.010 respectively) (Table 4).

Table 4 - Clinical outcome results and relation with covariates in the study, historical and overall period

Outcomes in relation with covariates	*2002-2003*	*1995-2001*	*Overall (1995-2003)*
Primary graft failure	Re-graft other eye (p=0.028)	No relation	Re-graft other eye (p=0.017)
Delayed epithelialisation of the graft	No relation	Surgeon (p=0.002)	Bank (p=0.007)
Graft clearness	Surgeon (p=0.047)	No relation	Bank (p=0.010)

The graft survival (2 years period) showed a difference in graft survival (p= 0.005) for the 5 eye banks (Figure 1).

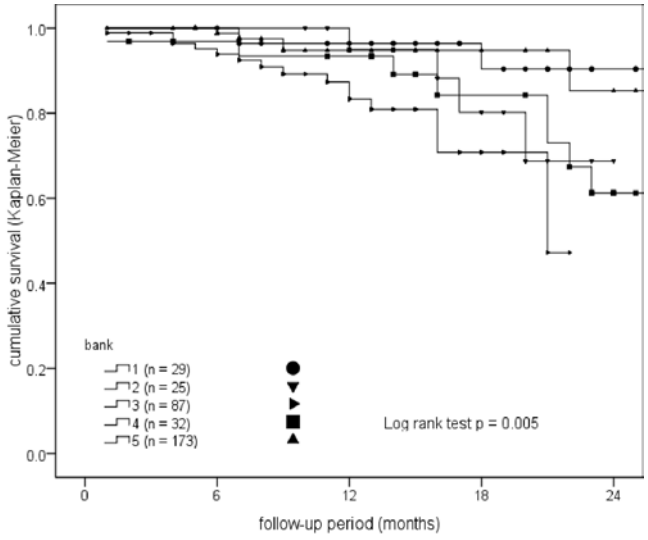

Figure 1: Graft survival (Kaplan Meier) of corneas processed by different eye banks.

Discussion

This study shows a poorer graft outcome in the study group (2002-2003), where different eye banks were involved in tissue processing compared to the historical control group (1995-2001), where only the local eye bank processed the tissue. The results suggest that the bank, processing the tissue is a risk factor for clinical outcome. This observation provides the scientific basis for the initial hesitations of the corneal surgeons about donor tissue quality evolving in rumours and finally in not accepting tissue from certain banks.

This study shows the controversy between the clinical non-acceptable proportion of events (e.g. 5 PF out of 173) and the scientifically requested numbers to reach statistical significance.

The incidence of primary failures (PF), a generally accepted serious adverse reaction [16,17,19-21] was 1.4% (5 PF on 346 grafts). This was below the 2.2% reported PF rates for hypothermic preservation,[19] but above those for OC (0- 0.4%) in single eye bank studies.[20,22,23] The fact that there were no primary failures in the control period precluded assessment of the odds ratio independent of the number of PF in the study group.

A remarkable high incidence of PF was observed with corneal tissue from bank 3. If we exclude this bank the incidence of PF of the remaining banks was within the reported limits. Clustering of PF was reported earlier.[24,25,26] Mead suggested the individual surgeon as the most important risk factor. This could not be confirmed by our study. Herpes infection as probable cause has also been reported.[25] Our observation of the eye bank as most important risk factor for PF confirmed the observation of Buxton.[26]

The incidence of delayed epithelialisation (DE) after grafting was significantly higher in the study group compared to the historical group. Analysis of the epithelial status of the grafts was possible at 9 days after transplantation for all grafts, for outpatients as well as for hospitalized patients. To our knowledge this study shows for the first time data on DE in OC preserved grafts, frequency varying from 2.9-24%, dependent on the bank (table 3). Clinical studies of epithelial healing after penetrating keratoplasty (PKP) are rare. Chou published about 84 penetrating grafts, stored in Optisol and reported 15% epithelial defects one week postoperatively.[27] Other studies, just as ours, showed a surgeon dependence pattern for epithelial healing.[28]

Delayed epithelialisation in hypothermically preserved donor corneas was described as a risk factor for long term graft survival.[9] This study and our results suggest that an aberrant frequency of DE may be considered as a serious adverse reaction.

In general surface complications in the first post operative year are known as leading causes for corneal graft failure[29-32] but they are mainly associated with recipient related causes.[30,32]

In concordance with the observations of the Australian Graft registry reporting on hypothermically stored donor corneas,[33] we observed an effect of different banks on graft survival with OC donor tissue. Some authors suggested that corneal transplantations with imported corneas are as safe and effective as those with domestic ones.[8,10] In contrast to our study the imported corneas were shipped form eye bank to eye bank and extensively evaluated on arrival and before surgery in the eye clinic.[8] A significant number of the imported corneas was discarded because of observed endothelial cell losses at arrival.9 Our study and the inconclusive results of the literature demonstrate that caution is warranted for imported donor tissue as long as conclusive information about processing and selection steps affecting graft outcome are lacking.

Donor related microbial infections were not observed in this study. The incidence of endophthalmitis reported after a properly performed OC procedure is 0–0.1%.[2] Microbiological safety of the tissue stored by organ culture was obtained by discarding contaminated tissue before grafting.[14]

Considerations and conclusions

Despite technical variations existed to suit local circumstances and preferences of corneal surgeons,[14] differences in clinical outcome for corneas from different banks were unexpected.

At one hand this study suggests that these variations affect graft outcome and this needs further investigation. Modifications in transport conditions have already been implemented.[1,2,33] At the other hand banks deviated from the routine procedure with other donor tissue and other than usual transplantation centres. This aspect might have been overlooked by all parties when help was sought for the processing of donor eyes retrieved in the Netherlands, kindly offered by foreign banks and gratefully accepted.

To improve and standardize results, in recent years European Union (EU) regulations (EU directives) have been implemented that made notification of serious adverse reactions (SARs) mandatory.[11,12,13]

This study demonstrates that a substantial number of cases and prolonged follow-up time are necessary to demonstrate changes in the outcome and consequently require improvement in the procedures.Each case considered as a SAR should be reported to the competent authorities. However, for detection of aberrant frequencies it is necessary to collect all cases in a National Graft Registry. Monitoring of these data by professionals and notification of deviations of these trends to the national competent authorities is essential and allows a consistent notification.Trust in eye banks should be based on control in the above suggested way.

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